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(57) Abstract

The invention relates to novel uses for inhibin, inhibin α subunit, activin inhibin or activin antagonists and compositions comprising them in the treatment or prevention of immune dysfunction and blood clotting disorders. Methods of treatment include administration of the required agent to a host, immunization of the host with the agent or passive immunization using antibodies raised against one of these agents.

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ACTIONS OF HORMONES

TECHNICAL FIELD

This invention relates to secondary uses for the gonadal hormones inhibin and activin and the α subunit of inhibin and to uses for antagonists of these hormones.

BACKGROUND ART

Inhibin is a gonadal glycoprotein which preferentially suppresses FSH secretion in vitro and is believed to have a key role in the physiological control of FSH in vivo. Recently, inhibin has been isolated from bovine (Robertson et al 1985 Biochem Biophys Res Comm 126:220; Robertson et al 1986, Mol Cell Endocrinol 44:271; Fukuda et al 1986 Mol Cell Endocrinol 44:55), porcine (Miyamoto et al 1985 Biochem Biophys Res Comm 129:396; Ling et al 1985, Proc Nat Acad Sci (USA) 82:7217) and ovine (Leversha et al 1987 J. Endocrinol 113:213) ovarian follicular fluids (FF). The amino acid sequence of porcine (Mason et al 1985 Nature 318:639), bovine (Forage et al 1986 Proc Nat Acad Sci (USA) 83:3091), human (Mason et al 1986 Biochem Biophys Res Comm 135:957; Stewart et al 1986 FEBS 206:329), ovine (Crawford et al 1987 Mol Endocrinol 1:699; Forage et al 1987 Serono Symposium 42:89) and rat (Esch et al 1987 Mol Endocrinol 1:388; Woodruff et al 1987 Mol Endocrinol 1:561) inhibin has been determined by cloning techniques. Inhibin is a dimer of two partially homologous subunits (α and β) joined by disulphide bonds. Inhibin with two variants of the β subunit (A and B) has been isolated from porcine FF (Ling et al 1985 Proc Nat Acad Sci (USA) 82:7217) and their mRNAs also identified in the human (Mason et al 1986 Biochem Biophys Res Comm 135:957; Stewart et al 1987 FEBS 206:329) and rat (Esch et al 1987 Mol Endocrinol 1:849; Woodruff et al 1987 Mol Endocrinol 1:561). Inhibin has also been isolated as two molecular weight forms (58000D and 31-32000D) which differ in the extent of processing of the α chain (Robertson et al 1986 Mol Cell Endocrinol 44:271). Bovine 58kD inhibin is cleaved to 31kD inhibin in the presence of serum but not follicular fluid (McLachlan et al 1986 Mol Cell Endocrinol 46:175) suggesting that processing of the α subunit is extragonadal. Inhibin has also been identified as a placental product in rats and humans (McLachlan et al 1986 Biochem Biophys Res Comm 140:485; Petraglia et al 1987 Science 237:187), suggesting that it plays a role in pregnancy.

Recently, several proteins have been isolated from gonadal and other tissues and found to be structurally related to inhibin particularly to the β subunit, but with different biological activities. These proteins

include activin-A and activin-AB (inhibin βAβA or βAβB subunit dimers, Vale et al 1986 Nature 321:776; Ling et al 1986 Nature 321:779) or erythroid differentiating factor (Eto et al 1987 Biochem Biophys Res Comm 142:1095); Mullerian inhibitory substance (Cate et al 1986, Cell 45:685) and transforming growth factor-β (Sporn et al 1987 J Cell Biol 105:1039). TGF-ß is a potent inhibitor of lectin-induced T lymphocyte proliferation in vitro, and many patients with glioblastomas, which secrete large amounts of TGF-ß, display suppressed immune functions (Kherl et al 1986 J Exp Med 153:1037; Wrann et al 1987 EMBO 6:1633).

DISCLOSURE OF THE INVENTION

Definitions

As used throughout the specification and claims "host" refers to a vertebrate host, preferably a mammalian host, and more particularly a human host.

The term "inhibin" as used throughout the specification is not restricted to inhibin isolated from a particular source and may include inhibin A ($\alpha\ \beta_A$) or inhibin B ($\alpha\ \beta_B$). The inhibin used may be naturally occurring or a recombinant or synthetic or semi/synthetic inhibin. It is recognised that for human applications the inhibin of choice would be a human inhibin whereas for veterinary applications one would preferably choose an inhibin derived from the appropriate animal species, unless an immune response is desired in which case an inhibin derived from a species differing from the host would be desirable. Purification and characterisation of native inhibin is described in PCT/AU85/00119. Recombinant inhibin is described in PCT/AU86/00097.

The term "activin" as used throughout the specification is not restricted to activin isolated from a particular source and may include activin A, activin AB or activin B ($\beta_B\ \beta_B$ dimer). The activin used may be naturally occurring or a recombinant or synthetic or semi/synthetic activin. It is recognised that for human applications the activin of choice would be a human activin whereas for veterinary applications one would preferably choose an activin derived from the appropriate animal species, unless an immune response is desired in which case an activin derived from a species differing from the host would be desirable.

The term "α subunit" as used throughout the specification and claims refers to the α subunit of inhibin including precursor forms. It is not restricted to α subunit derived from a particular source. The α subunit used may be naturally occurring or a recombinant or synthetic or

semi/synthetic α subunit. It is recognised that the α subunit of choice for inducing an immune response would be derived from a species differing from the host .

The term "inhibin composition" as used throughout the specification and claims refers to a composition comprising inhibin together with a pharmaceutically acceptable carrier, diluent, excipient and/or adjuvant.

The term "α subunit composition" as used throughout the specification and claims refers to a composition comprising inhibin α subunit together with a pharmaceutically acceptable carrier, diluent, excipient and/or adjuvant.

The term "activin composition" as used throughout the specification and claims refers to a composition comprising activin together with a pharmaceutically acceptable carrier, diluent, excipient and/or adjuvant.

The term "pharmaceutically acceptable" as used throughout the specification and claims extends to acceptability for human use or for other vertebrate use depending on whether the host, in a particular instance, is a human or other vertebrate.

The term "inhibin antagonist" refers to antagonists acting on the α subunit, β subunit or both and includes activin, TGF-β or other molecules having an activity or effect that is opposite to that of inhibin or that blocks the action of inhibin without necessarily combining with inhibin, antibodies to inhibin or to its α subunit or other molecules that neutralise the biological activities or effects of inhibin by combining with inhibin or with the α subunit of inhibin.

The term "inhibin antagonist composition" as used throughout the specification and claims refers to a composition comprising an inhibin antagonist and/or an antagonist of the α subunit of inhibin together with a pharmaceutically acceptable carrier, diluent, excipient and/or adjuvant.

The term "activin antagonist" as used throughout the specification and claims includes molecules having an activity or effect that is opposite to that of activin or that blocks or neutralizes the action of activin without necessarily combining with activin, as well as antibodies to activin or to its subunits, inhibin, and other inhibin-like molecules such as FSP, or follistatin [Uemo et al (1987) PNAS USA 84 8282; Robertson et al (1987) Biochem Biophys Res Comm 149 744; PCT/AU88/00024].

The term "activin antagonist composition" as used throughout the specification and claims refers to a composition comprising an activin antagonist together with a pharmaceutically acceptable carrier, diluent, excipient and/or adjuvant.

The term "adjuvant" as used throughout the specification refers, in respect to immunising compositions, to an agent used to enhance the immune response of the immunised host to the immunising composition. In respect of pharmaceutical compositions which are to be administered for therapeutic or prophylactic purposes other than immunisation, the term "adjuvant" refers to an agent used to enhance the desired therapeutic or prophylactic response of the host to the composition administered.

The term "antibody composition" as used throughout the specification and claims refers to a composition comprising the appropriate antibodies together with a pharmaceutically acceptable carrier, adjuvant, excipient and/or diluent.

The present invention relates to uses of the gonadal hormones inhibin and activin as well as the α subunit of inhibin and antagonists to inhibin and activin for purposes other than modulation of gonadal function.

The present invention demonstrates that bovine inhibin stimulates, while bovine activin suppresses lectin-induced proliferation of T-lymphocytes from rat thymus in vitro. Rabbits immunised with inhibin show a suppression of immunoglobulin levels compared to immunised and non-immunised control rabbits. These findings indicate that inhibin and activin are intimately involved in the regulation of immune responses. In contrast to its effects on T-lymphocytes, activin stimulates proliferation of 3T3 mouse embryo fibroblast cells in vitro but inhibin had no effect on these cells. These observations indicate that activin is a stimulatory growth factor and that the actions of inhibin and activin are cell-type specific.

Previous studies have shown that where the α subunit of inhibin is used as an immunogen, antibodies are generated that react with inhibin and neutralise its activity [Forage et al (1987) J. Endocrinol. 114 R1-R4: Findlay et al (1988) J. Endocrinology 119]. Therefore, inhibin and the α subunit of inhibin can be regarded at least as equivalent immunogens and the α subunit may be a superior immunogen since α subunit antibodies will neutralise both inhibin A and inhibin B and no β subunit antibodies can be generated that would cross-react with activin. Based on the above findings it follows that where immunisation against inhibin is required for immunoregulatory or blood clotting purposes, immunisation against inhibin α subunit can be utilised to achieve the same end.

Based on these observations, inhibin is suitable as:

1. an immunostimulatory agent for treatment of immunosuppression states and immunodeficiency diseases, including acquired immune deficiency syndrome (AIDS) and as an agent to stimulate the immune system response to infections and tumours.
2. an immunogen for treatment of autoimmune diseases (eg. systemic lupus erythematosus) and suppression of transplantation rejection responses. As explained above immunisation against the α subunit can also be used. It follows that other inhibin antagonists would be of use in the treatment of autoimmune diseases and for the suppression of transplantation rejection.
3. a diagnostic for monitoring the immune status of patients.

Based on these observations, activin is suitable as

1. an agent for the treatment of autoimmune diseases and for inhibition of transplantation rejection responses.
2. a growth promoter for treatment of wounds including surgical lesions, burns, tissue grafts and chronic ulcers.

From these observations it follows that activin antagonists can be used as:

1. inhibitors of excess tissue proliferation for the treatment of conditions such as keloid.
2. immunostimulants.
3. diagnostic agents for monitoring of immune status of patients and tumour growth.

Further, the inventors have found immunisation against both inhibin and the α subunit of inhibin to effect in vivo neutralization of inhibin results in more rapid clotting of blood.

These observations indicate that inhibin acts to delay clotting and may have widespread applications to delay thrombic processes.

Alternatively, antagonists to inhibin enhance the clotting process for treatment of haemorrhage and clotting disorders.

Based on these observations, inhibin is suitable as:

1. an agent for the delay of blood clotting and delay of thrombic processes.

Inhibin antagonists are suitable as:

1. agents to enhance the clotting process; and
2. agents for the treatment of haemorrhage and clotting disorders.

Inhibin or the α subunit thereof can be used as immunogens to

effect more rapid blood clotting.

The invention provides a method for the treatment of immunosuppressive states or immunodeficiency diseases in a host in need of such treatment which method comprises administering an effective amount of inhibin or an inhibin composition, to said host.

Immunosuppressive states to be treated include acquired immune deficiency syndrome (AIDS).

The invention also provides a method of stimulating the immune response of a host in need of such treatment, to an infection or a tumour which method comprises administering an immunostimulatory amount of inhibin or an inhibin composition, to said host.

The invention further provides a method for the treatment of an autoimmune disease in a host in need of such treatment which method comprises immunising said host with an autoimmune disease treating amount of inhibin, the α subunit of inhibin, an α subunit composition or an inhibin composition. Alternatively, the host may be passively immunised with inhibin antibodies, α subunit antibodies, or an inhibin antibody or α subunit antibody composition. The method may comprise administering an autoimmune disease treating amount of an inhibin antagonist or inhibin antagonist composition to the host.

In another embodiment, the invention provides a method of suppressing transplantation rejection in a host in need of such treatment which method comprises immunising said host with a transplantation rejection suppressing amount of inhibin, the α subunit of inhibin or an inhibin or α subunit composition. Alternatively, the host may be passively immunised with inhibin antibodies including α subunit antibodies or with an inhibin or α subunit antibody composition. The method may comprise administering a transplantation rejection suppressing amount of an inhibin antagonist or an inhibin antagonist composition to the host.

In yet another embodiment, the invention provides a method for monitoring the immune status of a host in need of such treatment which method comprises measuring the ability of said host to respond to immunostimulation by inhibin or an inhibin composition.

The invention also provides a method for treating an autoimmune disease in a host in need of such treatment which method comprises administering to said host an autoimmune disease treating amount of activin or an activin composition.

In another embodiment, the invention provides a method of inhibiting

a transplantation rejection response in a host in need of such treatment which method comprises administering a transplantation rejection suppressing amount of activin or an activin composition, to said host.

The invention also provides a method for treating a wound including a surgical lesion, burn, tissue graft or chronic ulcer in a host in need of such treatment which method comprises administering to said host a growth promoting amount of a growth promoter comprising activin or an activin composition.

The invention further provides a method of monitoring the immune status of a host in need of such treatment which method comprises measuring the levels of inhibin and/or activin in serum or tissues by radioimmunoassay or bioassay.

The invention also provides a method of monitoring tumour growth in a host in need of such treatment which method comprises measuring the levels of activin in serum or tissues by radioimmunoassay or bioassay.

The invention further provides a method for inhibiting excess tissue proliferation in a host in need of such treatment which method comprises administering an effective amount of an activin antagonist or an activin antagonist composition to said host. This method would extend to immunisation against activin.

The invention still further provides a method of stimulating the immune response of a host in need of such treatment which method comprises administering an immunostimulatory amount of an activin antagonist or of an activin antagonist composition to a host in need of such treatment.

The invention provides a method of enhancing blood clotting in a host in need of such treatment which method comprises immunising said host with an immunising amount of inhibin or the α subunit of inhibin or an inhibin or α subunit composition. Alternatively, the host may be passively immunised with inhibin or α subunit antibodies or a composition comprising such antibodies.

Enhancement of blood clotting can also be achieved by administration of a blood clotting enhancing amount of at least one inhibin antagonist or an antagonist composition, to the host.

The invention also provides a method of inhibiting blood clotting in a host in need of such treatment which method comprises administration of a blood clotting inhibiting amount of inhibin or an inhibin composition to said host.

The invention includes a method of delaying thrombic processes in a

host in need of such treatment, which method comprises administering a thrombic process delaying amount of inhibin or an inhibin composition to said host.

The invention also provides a method of treating haemorrhaging which method comprises administering a haemorrhaging treating amount of an inhibin antagonist or an antagonist composition to said host, including inhibin or α subunit antibodies. Haemmorhaging could also be treated by immunising the host with inhibin, the α subunit of inhibin or with an inhibin or α subunit composition.

The invention provides vaccines for immunising hosts

1. against an autoimmune disease,
2. to suppress transplant rejection,
3. to enhance blood clotting,
4. to prevent haemorrhaging

comprising inhibin, α subunit of inhibin, an inhibin composition, or an α subunit composition.

The invention also provides a method of using inhibin in the preparation of a pharmaceutical composition for the delaying of thrombic processes.

The invention provides a method of using inhibin in the preparation of a pharmaceutical composition for the treatment of immunosuppressive states or immunodeficiency diseases.

The invention also provides a method of using inhibin in the preparation of a pharmaceutical composition for stimulating the immune response of a host to an infection or a tumour.

The invention further provides a method of using inhibin or inhibin α subunit in the preparation of an immunogen for protecting a host against an autoimmune disease.

The invention also provides a method of using an inhibin antagonist in the preparation of a pharmaceutical composition for the treatment of an autoimmune disease.

In another form, the invention provides a method of using inhibin or inhibin α subunit in the preparation of an immunogen for protecting a host against a transplantation rejection response.

The invention also provides a method of using an inhibin antagonist in the preparation of a pharmaceutical composition for the treatment of a transplantation rejection response.

In yet another form the invention provides a method of using inhibin

in the preparation of a diagnostic agent for monitoring the immune status of a host.

The invention provides a method of using activin for the preparation of a pharmaceutical composition for the treatment of an autoimmune disease.

The invention also provides a method of using activin for the preparation of a pharmaceutical composition for suppressing transplant rejection.

The invention further provides a method of using activin for the preparation of pharmaceutical composition for promotion of tissue regeneration in a wound including a surgical lesion, burn, tissue graft or chronic ulcer.

In a further embodiment the invention provides a method of using activin for the preparation of a pharmaceutical composition for monitoring the immune status of a host.

The invention provides a method of using activin for the preparation of a pharmaceutical composition for monitoring tumour growth in a host.

In another form, the invention provides a method of using an activin antagonist for the preparation of a pharmaceutical composition for inhibiting excessive tissue proliferation.

The invention also provides a method of using inhibin or the α subunit of inhibin for the preparation of an immunogen for enhancing blood clotting.

The invention further provides a method of using an inhibin antagonist for the preparation of a pharmaceutical composition for enhancing blood clotting.

The invention provides a method of using inhibin for the preparation of a pharmaceutical composition for inhibiting blood clotting.

The invention also provides a method of using inhibin for the preparation of a pharmaceutical composition for delaying thrombic processes.

The invention further provides a method of using inhibin or the α subunit thereof for the preparation of an immunogen for preventing haemorrhaging.

The invention also provides a method of using an inhibin antagonist for the preparation of a pharmaceutical composition for the treatment of haemorrhaging.

For those methods of the invention which comprise passive immunisation the invention provides inhibin antibodies, α subunit antibodies or compositions comprising inhibin or α subunit antibodies to be used as passive vaccines.

The amount of inhibin, activin, inhibin α subunit, inhibin antagonist or activin antagonist that may be combined with carrier to produce a single dosage form will vary depending upon the host to be treated and the particular mode of administration.

It will be understood, also, that the specific dose level for any particular patient will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, sex, diet, time of administration, route of administration, rate of excretion, drug combination, the particular immunoregulatory or blood clotting state being treated and the severity of the particular condition undergoing treatment.

The compositions of the present invention may be administered orally, parenterally, by inhalation spray, rectally, vaginally or topically in dosage unit formulations containing conventional, non-toxic, pharmaceutically acceptable carriers, diluents, adjuvants and/or excipients as desired.

The term parenteral as used herein includes subcutaneous injections, intravenous, or intramuscular injection, or infusion techniques.

Injectable preparations, for example, sterile injectable aqueous or oleagenous suspensions may be formulated according to the known art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally acceptable diluent or solvent, for example, as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution, and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables.

The term "pharmaceutically acceptable adjuvant" can mean either the standard compositions which are suitable for human administration or the typical adjuvants employed in animal vaccinations.

Suitable adjuvants for the vaccination of animals and humans include but are not limited to oil emulsions such as Freund's complete or incomplete adjuvant (not suitable for human or livestock use), Marcol 52: Montanide 888 (Marcol is a Trademark of Esso. Montanide is a Trademark of SEPPIC, Paris), squalane or squalene, Adjuvant 65 (containing peanut oil,

mannide monooleate and aluminum monostearate), mineral gels such as aluminum hydroxide, aluminum phosphate, calcium phosphate and alum, surfactants such as hexadecylamine, octadecylamine, lysolecithin, dimethyldioctadecylammonium bromide, N,N-dioctadecyl-N',N'-bis(2-hydroxyethyl)propanediamine, methoxyhexadecylglycerol and pluronic polyols, polyanions such as pyran, dextran sulfate, polyIC, polyacrylic acid and carbopol, peptides and amino acids such as muramyl dipeptide, dimethylglycine, tuftsin and trehalose dimycolate. The active substances of the present invention can also be administered following incorporation into liposomes or other micro-carriers, or after conjugation to polysaccharides, proteins or polymers or in combination with Quil-A to form "Iscoms" (immunostimulating complexes) (Morein et al., Nature 308, 457-460 [1984]). Other adjuvants suitable for use in the present invention include fusion proteins or conjugates comprising the active compound of interest together with an integral membrane protein of prokaryotic or eukaryotic origin, such as TraT.

Routes of administration, dosages to be administered as well as frequency of injections are all factors which can be optimized using ordinary skill in the art. Typically, the initial vaccination is followed some weeks later by one or more "booster" vaccinations, the net effect of which is the production of high titres of antibodies against the immunogen.

Suppositories for rectal or vaginal administration of the compositions of the invention can be prepared by mixing the composition with a suitable nonirritating excipient such as cocoa butter, theobroma oil, glycerinated gelatin or polyethylene glycols which are solid at ordinary temperatures but liquid at the rectal or vaginal temperature or by contact with fluids present in the appropriate cavity and will therefore melt in the rectum or vagina and release the drug.

Compositions for topical administration include creams, ointments and pastes. The ingredients that constitute the base of ointments (e.g. petrolatum, waxes) are melted together; powdered drug components are added and the mass stirred with cooling. Generally, the product is then passed through a roller mill to achieve the particle-size range desired for the dispersed solid. Pastes are ointments with relatively large, dispersed solid content, and are prepared similarly.

Creams are semisolid emulsions, either water-in-oil or oil-in-water. A solid ingredient can be added to the appropriate phase before emulsification or may be dispersed at some point after the emulsification

step. Topical dosage forms include disc dosage form systems that have been used for the transdermal delivery of therapeutic agents. They provide uniform and prolonged drug release.

Solid dosage forms for oral administration may include capsules, tablets, pills, powders, and granules. In such solid dosage forms, inhibin activin, α subunit or antagonists may be admixed with at least one inert diluent such as sucrose, lactose or starch. Such dosage forms may also comprise, as is normal practice, additional substances other than inert diluents, e.g., lubricating agents such as magnesium stearate. In the case of capsules, tablets, and pills, the dosage forms may also comprise buffering agents. Tablets and pills can additionally be prepared with enteric coatings.

Liquid dosage forms for oral administration may include nanoparticles, microcapsules, LTB conjugates, cholera or its B subunit as a conjugate, or vitamin B12 conjugates in pharmaceutically acceptable emulsions, syrups, solutions, suspensions, and elixirs containing inert diluents commonly used in the art, such as water. Such compositions may also comprise adjuvants, such as wetting agents, emulsifying and suspending agents or TraT as a conjugate, and sweetening, flavoring, and perfuming agents including sugars such as sucrose, sorbitol, fructose etc, glycols such as polyethylene glycol, propylene glycol etc, oils such as sesame oil, olive oil, soybean oil etc, antiseptics such as alkylparahydroxybenzoate etc, and flavours such as strawberry flavour, peppermint etc.

An advantage of the therapeutic methods disclosed herein is that the agents to be used are natural products which have been shown to play a physiological role.

Such an approach is of use in avoiding cytotoxicity problems experienced with available drugs.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1A shows the stimulation of tritiated thymidine incorporation into rat T-lymphocytes in vitro by the non-specific mitogens Concanavalin A (Con A) and phytohaemagglutinin (PHA). Values are mean ± SEM (n = 4 replicates).

Figure 1B shows the stimulation of tritiated thymidine incorporation into rat T-lymphocytes by bovine 31kD inhibin in the absence and presence of submaximal doses of Con A (0.125μg/ml) or PHA (50μg/ml). There was no effect of inhibin in the presence of maximally stimulating dose (1μg/ml) of Con A. Values are mean ± SEM (n = 4 replicates).

Figure 2 shows the suppression by porcine TGF-ß of tritiated thymidine incorporation into rat T-lymphocytes in the absence and presence of a maximal dose of Con A (1µg/ml). Values are mean ± SEM (n = 4 replicates). *p < 0.05 compared with control.

Figure 3 shows inhibition of tritated thymidine incorporation into rat T-lymphocytes by bovine activin and TGF-ß in the presence of a submaximal dose of PHA (50µg/ml).

Figure 4 shows stimulation by bovine activin and porcine TGF-ß of tritiated thymidine incorporation into mouse 3T3 cells in vitro. Bovine inhibin had no effect in this system. Values are mean ± SEM (n = 4 replicates).

Figure 5 shows profiles of serum IgG levels and inhibin-specific or control protein-specific antibody titre in rabbits receiving multiple immunisations with bovine inhibin (Fig 5A) and control protein (Fig 5B). NS: regression is not significant.

Figure 6 shows the binding of sheep antibodies, as serum diluted 1:500, to iodinated 31kD bovine inhibin. The sera are grouped as high responders (≥ 1% binding) and low responders (< 1% binding) and controls (not vaccinated with active ingredient). The data show that there is a significant difference between the high responders and the other two groups ($P < 0.001$; students t-test). Data are the mean ± sem; number of animals per group is as shown in Figure 7.

Figure 7 shows the clotting time (in seconds) between the serum groups shown in Figure 6. The data show that there is a significant difference between the high responders and the other two groups ($P < 0.001$; students t-test). Data are the mean ± sem; n = number of animals per group.

BEST MODE AND OTHER MODE(S) FOR CARRYING OUT THE INVENTION

In the method for the treatment of immunosuppressive states or immunodeficiency diseases, an effective amount of inhibin or an inihibin composition is administered to the host.

The inhibin composition for this purpose is prepared by mixing, preferably homogenously mixing, inhibin with a pharmaceutically acceptable carrier, diluent, excipient and/or adjuvant using standard methods of pharmaceutical preparation.

The amount of inhibin required to produce a single dosage form will vary depending upon the host to be treated and the particular mode of administration. The specific dose level for any particular patient will depend upon a variety of factors including the activity of the specific

inhibin employed, the age, body weight, general health, sex, and diet of the patient, time of administration, route of administration, rate of excretion, drug combination and the severity of the immunosuppressive state or immunodeficiency disease undergoing treatment.

Inhibin or inhibin compositions for use in the treatment of immunosuppressive states or immunodeficiency diseases include those to be administered orally, parenterally, by inhalation spray, rectally, vaginally or topically in unit dosage formulations containing conventional, non-toxic, pharmaceutically acceptable carriers, diluents, excipients and/or adjuvants as desired.

In the method for stimulating the immune response of a host to an infection or to a tumour, an immune response stimulating amount of inhibin or an inihibin composition is administered to the host.

The inhibin composition for this purpose is prepared by mixing, preferably homogenously mixing, inhibin with a pharmaceutically acceptable carrier, diluent, excipient and/or adjuvant using standard methods of pharmaceutical preparation.

The amount of inhibin required to produce a single dosage form will vary depending upon the host to be treated and the particular mode of administration. The specific dose level for any particular patient will depend upon a variety of factors including the activity of the specific inhibin employed, the age, body weight, general health, sex, and diet of the patient, time of administration, route of administration, rate of excretion, drug combination and the severity of the infection or tumour undergoing treatment.

Inhibin or inhibin compositions for use in the stimulation of an immune response to an infection or tumour include those to be administered orally, parenterally, by inhalation spray, rectally, vaginally or topically in unit dosage formulations containing conventional, non-toxic, pharmaceutically acceptable carriers, diluents, excipients and/or adjuvants as desired.

In the method for treatment of autoimmune disease, the host is immunised with an autoimmune disease treating amount of inhibin or an inhibin composition, α subunit or an α subunit composition. Immunisation may also be passive immunisation with the host receiving inhibin antibodies or an antibody preparation comprising antibodies raised against inhibin or the α subunit of inhibin. The antibodies are raised by immunising a host capable of raising an immune response to inhibin with

inhibin, α subunit, or an inhibin or α subunit composition, and administered either as antibodies or as an antibody composition. Alternatively, an autoimmune disease treating amount of activin or an activin composition or other inhibin antagonist or other inhibin antagonist composition is administered to the host.

The inhibin composition, activin composition, α subunit composition, inhibin antagonist composition or antibody composition for this purpose is prepared by mixing, preferably homogenously mixing, inhibin, activin, α subunit, inhibin antagonist or inhibin or α subunit antibodies respectively with a pharmaceutically aceptable carrier, diluent, excipient and/or adjuvant using standard methods of pharmaceutical preparation.

The amount of inhibin, α subunit, inhibin antagonist, activin, or inhibin or α subunit antibodies required to produce a single dosage form will vary depending upon the host to be treated and the particular mode of administration. The specific dose level for any particular patient will depend upon a variety of factors including the activity of the specific molecule employed, the age, body weight, general health, sex, and diet of the patient, time of administration, route of administration, rate of excretion, drug combination and the severity of the autoimmune disease undergoing treatment.

Inhibin, α subunit, inhibin or α subunit compositions, activin, activin compositions, inhibin antagonist or inhibin antagonist compositions for use in the treatment of autoimmune diseases include those to be administered orally, parenterally, by inhalation spray, rectally, vaginally or topically in unit dosage formulations containing conventional, non-toxic, pharmaceutically acceptable carriers, diluents, excipients and/or adjuvants as desired. Antibodies or antibody compositions are administered parenterally. In newborns they may also be administered orally.

In the method for suppressing transplantation rejection the host is immunised with a transplantation rejection suppressing amount of inhibin, an inhibin composition, α subunit or an α subunit composition. Immunisation may also be passive immunisation with the host receiving an antibody preparation comprising antibodies raised against inhibin or inhibin α subunit. The antibodies are raised by immunising a host capable of raising an immune response to inhibin with inhibin or an inhibin composition or α subunit or an α subunit composition, and administered

either as antibodies or as an antibody composition. Alternatively, transplantation rejection may be suppressed by administering a transplantation rejection suppressing amount of activin or an activin composition or an inhibin antagonist or an inhibin antagonist composition to the host.

The inhibin, α subunit, activin, inhibin antagonist or antibody composition for this purpose is prepared by mixing, preferably homogenously mixing, inhibin, α subunit, activin, inhibin antagonist or antibodies respectively with a pharmaceutically acceptable carrier, diluent, excipient and/or adjuvant using standard methods of pharmaceutical preparation.

The amount of inhibin, α subunit, activin, inhibin antagonist or antibody required to produce a single dosage form will vary depending upon the host to be treated and the particular mode of administration. The specific dose level for any particular patient will depend upon a variety of factors including the activity of the specific inhibin employed, the age, body weight, general health, sex, and diet of the patient, time of administration, route of administration, rate of excretion, drug combination and the severity of the transplantation rejection undergoing treatment.

Inhibin, α subunit or inhibin or α subunit compositions or inhibin antagonist or inhibin antagonist compositions or activin or activin compositions for use in the suppression of transplantation rejection include those to be administered orally, parenterally, by inhalation spray, rectally, vaginally, or topically in unit dosage formulations containing conventional, non-toxic, pharmaceutically acceptable carriers, diluents, excipients and/or adjuvants as desired. Inhibin antibodies, α subunit antibodies, or inhibin antibody compositions or α subunit antibody compositions are administered parenterally. In newborns they may also be administerd orally.

In the method for monitoring the immune status of a host, the patient is treated with an immunostimulatory dose of inhibin and subsequently monitored for the ability to respond by changes in immune system parameters, particularly lymphocyte numbers and immunoglobulin levels in the circulating blood. A poor response would be indicative of imunodeficiency. Serum or biopsy tissue from organs of the immune system of a patient are collected and assayed for inhibin or activin levels by radioimmunoassay or bioassay. Low levels of inhibin or high levels of activin would indicate immunodeficiency.

In the method for inhibiting excessive tissue proliferation an effective amount of an activin antagonist or an activin antagonist composition is administered to the host.

The activin antagonist composition for this purpose is prepared by mixing, preferably homogenously mixing, an activin antagonist with a pharmaceutically acceptable carrier, diluent, excipient and/or adjuvant using standard methods of pharmaceutical preparation.

The amount of activin antagonist required to produce a single dosage form will vary depending upon the host to be treated and the particular mode of administration. The specific dose level for any particular patient will depend upon a variety of factors including the activity of the specific inhibin employed, the age, body weight, general health, sex, and diet of the patient, time of administration, route of administration, rate of excretion, drug combination and the severity of the excessive tissue proliferation undergoing treatment.

Activin antagonists or activin antagonist compositions for use in the inhibition of excessive tissue proliferation include those to be administered orally, parenterally, by inhalation spray, rectally, vaginally, or topically in unit dosage formulations containing conventional, non-toxic, pharmaceutically acceptable carriers, diluents, excipients and/or adjuvants as desired.

In the method for promoting tissue regeneration in a wound including a surgical lesion, burn, tissue graft or chronic ulcer, a tissue regeneration promoting amount of activin or an activin composition is administered to the host.

The activin composition for this purpose is prepared by mixing, preferably homogenously mixing, activin with a pharmaceutically acceptable carrier, diluent, excipient and/or adjuvant using standard methods of pharmaceutical preparation.

The amount of activin required to produce a single dosage form will vary depending upon the host to be treated and the particular mode of administration. The specific dose level for any particular patient will depend upon a variety of factors including the activity of the specific inhibin employed, the age, body weight, general health, sex, and diet of the patient, time of administration, route of administration, rate of excretion, drug combination and the severity of the wound, burn, tissue graft or chronic ulcer undergoing treatment.

Activin or activin compositions for use in the promotion of tissue

regeneration include those to be administered orally, parenterally, by inhalation spray, rectally, vaginally, or topically in unit dosage formulations containing conventional, non-toxic, pharmaceutically acceptable carriers, diluents, excipients and/or adjuvants as desired.

In the method for monitoring tumour growth serum or tumour biopsy tissue is collected from a patient and assayed for activin levels by radioimmunoassay or bioassay. High levels would be indicative of tumour growth.

In the method for enhancing blood clotting, the host is immunised with inhibin, the α subunit of inhibin or an inhibin or α subunit composition. Immunisation may also be passive immunisation with the host receiving an antibody preparation comprising antibodies raised against inhibin or α subunit. The antibodies are raised by immunising a host capable of raising an immune response to inhibin, α subunit or an inhibin or α subunit composition, and administered either as antibodies or as an antibody composition. Alternatively, a blood clot enhancing amount of an inhibin antagonist or an inhibin antagonist composition is administered to the host.

The inhibin, α subunit, inhibin antagonist or antibody composition for this purpose is prepared by mixing, preferably homogenously mixing, inhibin, α subunit or an inhibin antagonist or inhibin or α subunit antibodies respectively with a pharmaceutically acceptable carrier, diluent, excipient and/or adjuvant using standard methods of pharmaceutical preparation.

The amount of inhibin, α subunit, inhibin antagonist, or antibodies required to produce a single dosage form will vary depending upon the host to be treated and the particular mode of administration. The specific dose level for any particular patient will depend upon a variety of factors including the activity of the specific inhibin employed, the age, body weight, general health, sex, and diet of the patient, time of administration, route of administration, rate of excretion, drug combination and the severity of the clotting condition undergoing treatment.

The inhibin, α subunit, antibody or antagonist compositions for use in the enhancement of blood clotting include those to be administered orally, parenterally, by inhalation spray, rectally, vaginally, or topically in unit dosage formulations containing conventional, non-toxic, pharmaceutically acceptable carriers, diluents, excipients and/or adjuvants as desired. Antibodies or antibody compositions are administered parenterally. In newborns they may also be administered orally.

In the method for inhibiting blood clotting, inhibin, or an inhibin composition is administered to the host.

The inhibin composition for this purpose is prepared by mixing, preferably homogenously mixing, inhibin or α subunit respectively with a pharmaceutically acceptable carrier, diluent, excipient and/or adjuvant using standard methods of pharmaceutical preparation.

The amount of inhibin required to produce a single dosage form will vary depending upon the host to be treated and the particular mode of administration. The specific dose level for any particular patient will depend upon a variety of factors including the activity of the specific inhibin employed, the age, body weight, general health, sex, and diet of the patient, time of administration, route of administration, rate of excretion, drug combination and the severity of the clotting condition undergoing treatment.

The inhibin compositions for use in the inhibition of blood clotting include those to be administered orally, parenterally, by inhalation spray, rectally,vaginally, or topically in unit dosage formulations containing conventional, non-toxic, pharmaceutically acceptable carriers, diluents, excipients and/or adjuvants as desired.

In the method for delaying thrombic processes, inhibin, or an inhibin composition is administered to the host.

The inhibin composition for this purpose is prepared by mixing, preferably homogeneously mixing, inhibin or α subunit respectively with a pharmaceutically acceptable carrier, diluent, excipient and/or adjuvant using standard methods of pharmaceutical preparation.

The amount of inhibin required to produce a single dosage form will vary depending upon the host to be treated and the particular mode of administration. The specific dose level for any particular patient will depend upon a variety of factors including the activity of the specific inhibin employed, the age, body weight, general health, sex, and diet of the patient, time of administration, route of administration, rate of excretion, drug combination and the severity of the thrombic condition undergoing treatment.

The inhibin compositions for use in the delay of thrombic processes include those to be administered orally, parenterally, by inhalation spray, rectally, vaginally, or topically in unit dosage formulations containing conventional, non-toxic, pharmaceutically acceptable carriers, diluents, excipients and/or adjuvants as desired.

In the method for treatment of haemorrhaging an antagonist of inhibin or an inhibin antagonist composition is administered to the host. Alternatively the host may be immunised with inhibin or the α subunit of inhibin or an inhibin or α subunit composition or passively immunised with inhibin or α subunit antibodies or compositions comprising inhibin or α subunit antibodies.

The inhibin antagonist, inhibin, α subunit or antibody compositions for this purpose are prepared by mixing, preferably homogenously mixing, an inhibin antagonist, inhibin, α subunit, or antibodies respectively with a pharmaceutically acceptable carrier, diluent, excipient and/or adjuvant using standard methods of pharmaceutical preparation.

The amount of inhibin antagonist, inhibin, α subunit, or antibody required to produce a single dosage form will vary depending upon the host to be treated and the particular mode of administration. The specific dose level for any particular patient will depend upon a variety of factors including the activity of the specific inhibin employed, the age, body weight, general health, sex, and diet of the patient, time of administration, route of administration, rate of excretion, drug combination and the severity of the haemorrhaging to be treated.

The antagonist compositions for use in the treatment of haemorrhaging include those to be administered orally, parenterally, by inhalation spray, rectally, vaginally, or topically in unit dosage formulations containing conventional, non-toxic, pharmaceutically acceptable carriers, diluents, excipients and/or adjuvants as desired. Inhibin antibodies, α subunit antibodies, or inhibin antibody compositions or α subunit antibody compositions are administered parenterally. In newborns they may also be administerd orally.

EXAMPLE 1

Effect of inhibin and activin on the proliferation of rat T-lymphocytes in vitro

A conventional T-lymphocyte proliferative assay was employed as follows;

Thymuses from 60-70 d Sprague-Dawley male rats were excised under sterile conditions, the capsule cut and T-lymphocytes recovered mainly from the cortical region by manipulation of the tissue with blunt probes. The cells were washed in Dulbecco's Modified Eagle's Medium (DMEM) and plated (0.8 million cells/well) in 96 well tissue culture plates in the presence of test substances in a final volume of 250µl DMEM containing 5% fetal

calf serum (FCS). Cells were incubated at 37°C for 72 hr at which time tritiated thymidine (50µl, 0.5µCi/well, 6.7Ci/mmol) was added and the cells incubated a further 16-20hrs at 37°C.

The media containing the cells was collected, the cells washed with 10mM phosphate pH7-buffered 0.9% NaCl. 10% trichloroacetic acid (TCA, 1ml) was added and the precipitate collected by centifugation. The precipitate was washed with 10% TCA (1ml) followed by methanol (1ml). The resulting precipitate was dissolved in 0.3M NaOH (100µl) for 10 min at room temperature. 0.3M hydrochloric acid (100µl) was added and the sample counted by liquid scintillation in a γ counter.

Inhibin and activin were purified from bovine follicular fluid by procedures already described (Robertson et al 1985 Biochem Biophys Res Comm 126:220, Robertson et al 1986 Mol Cell Endocrinol 44:271; McLachlan et al 1987 J Clin Endocrinol Metab 65:954).

The results in Fig. 1 show that in the absence of Con A and PHA or in the presence of a submaximal dose of Con A (0.125µg/ml), inhibin showed a small but significant ($p < 0.05$) stimulation of thymidine incorporation by T-lymphocytes only at the highest dose employed (5nM). However, in the presence of 50µg/ml PHA, inhibin stimulated ($p < 0.001$) thymidine uptake at all doses above 0.5 nM with a plateau of stimulation observed at 2.5nM (ED50 0.7nM). Inhibin had no detectable effect on T lymphocytes maximally stimulated by Con A.

TGF-ß inhibited uptake by lymphocytes maximally-stimulated by Con A, with an ID50 of 0.02nM (Fig.2). Although bovine activin showed no effect in this experiment, other studies (data not shown) showed that the highest dose of activin (10nM) can suppress maximally stimulated lymphocytes indicating a variable effect of activin at high doses. There was no effect of activin up to 10nM on unstimulated lymphocytes. Both TGF-ß and activin inhibited PHA-stimulated (50µg/ml) thymidine uptake by T lymphocytes, with ID50 values of 0.004nM and 0.4nM, respectively (Figure 3).

EXAMPLE 2

The effect of inhibin and activin on the proliferation of a mouse 3T3 fibroblastic cell line

Mouse Balb/c 3T3 embryo fibroblasts were maintained at 37°C in DMEM supplemented with glucose (4.5g/l) and 10% FCS. Monolayers of these cells were trypsinised and the released cells were suspended in DMEM containing 10%FCS at a concentration of 50,000/ml. Samples of 200µl were placed into 96 well culture plates and incubated for 4 days. The medium was replaced

with DMEM containing 0.5% FCS. Cells were then incubated at 37°C for 20 hr at which time tritiated thymidine (10µl,0.4µCi/well, 6.7 Ci/mmole) was added and the cells incubated a further 2hrs at 37°C. The cells were washed with distilled water, 5% trichloroacetic acid (TCA, 0.2ml) added and the precipitate was washed with 10% TCA. The precipitate was dissolved in 0.3M NaOH (100µl) for 10 min at room temperature. Hydrochloric acid (100µl,0.3M) was added and the sample counted by liquid scintillation in a γ counter.

In contrast to the responses observed for T-lymphocytes in vitro, inhibin did not stimulate the proliferation of 3T3 cells, up to a maximum dose of 30nM (Fig. 4). However, activin stimulated 3T3 cell proliferation at doses above 3nM, but TGF-β stimulated proliferation even at the lowest dose employed (0.3nM).

EXAMPLE 3

IgG Levels in sera from inhibin-immunised and control rabbits

Since inhibin is a natural stimulator of the immune system, removal or neutralisation of inhibin by direct immunisation could result in lower serum IgG levels due to the neutralisation per se and to inhibitory effects of the remaining activin and/or TGF-β. To test this intact New Zealand male rabbits were used. Sera from a pool of normal rabbits and serum from a rabbit immunised against bovine 31kD inhibin were collected separately and immunoglobulin (IgG) fraction isolated following an inital 45% ammonium sulphate precipitation step and a fractionation step on Protein A immobolised to Sephacryl S200.

From 20ml serum, 38.2mg IgG was recovered from control animals compared with 23.4mg from the immunised animal. Details of the immunisation procedure and characterization of this antiserum have been previously described (McLachlan et al 1986 Mol Cell Endocrinol 46:175).

In further studies, serum IgG levels were determined by sandwich enzyme-linked immunosorbent assay. In this assay the solid phase was coated with sheep anti-rabbit IgG and incubated with either rabbit IgG standard or rabbit sera unknowns at multiple dilutions. The level of bound IgG in the standard or sample was detected with peroxidase-labelled goat anti-rabbit IgG. Serum IgG levels were determined in 3 rabbits receiving multiple immunisations with bovine 58 or 31kD inhibin and two rabbits receiving multiple injections with another protein structurally unrelated to inhibin and showing no effect on thymocyte proliferation (control protein). The antibody titre was assessed from binding of the respective iodinated

protein to serum at a final dilution of 1:6000. Examples of the changes in serum IgG and inhibin antibody titre following immunisation with inhibin or control protein is shown in Figure 5.

In order to analyse this relationship, the change in serum IgG levels after each secondary immunisation was determined from the significance of the regression of the IgG levels during the periods when the antibody titre was above 5 and 10% binding, respectively. As seen in Table 1, in the inhibin-immunised rabbits, 3 of 9 immunisations which resulted in antibody titres >10% showed a significant decrease in serum IgG, the remainder showing no change. In contrast, in control animals two immunisations caused an increase in IgG levels while two showed no change. It is concluded that inhibin immunisation can cause a suppression in serum IgG levels.

The results of Examples 1-3 indicate that inhibin stimulates the immune system at physiological doses, at least in part by direct stimulation of lymphocyte function as well as by enhancing the response of lymphocytes to mitogenic, and presumably antigenic, stimulation. In accordance with these observations in vitro, blocking endogenous inhibin action in vivo by passive immunisation results in a reduced capacity for immunoglobulin production. The immunostimulatory actions of inhibin is specific, as inhibin had no effect on the proliferation of 3T3 cells in vitro.

In contrast to the effects of inhibin, activin suppresses lymphocyte function at physiological doses, although at higher doses than observed with TGF-ß. Interestingly, the action of activin on 3T3 cell proliferation in vitro was opposite to its effects on the lymphocytes, being stimulatory at physiological doses. It is also postulated that inhibin and activin are also involved in regulating the unique immunologically privileged environments of the gonads and pregnant uterus, as well as growth regulation. Consequently, inhibin and activin have applications as therapeutic and diagnostic agents in a broad range of clinical conditions related to immune dysfunction and tissue growth promotion.

TABLE 1: Changes in IgG levels after booster immunisation with bovine inhibin or control protein

		No. of immunisations resulting in a change or no change in serum IgG levels			
Immunogen	Titre	Increase	No Change	Decrease	Total
Inhibin	<5%	1	0	0	1
	5-10%	0	3	0	3
	>10%	0	6	3	9
Control Protein	<10%	0	0	0	0
	>10%	2	2	0	4

For methodological details see text.

EXAMPLE 4

Effects of Inhibin Immunisation on Clotting Time in Adult Male Castrate Rabbits

Two male adult castrate New Zealand rabbits, aged approximately 2 years (#749 and #1989) were immunised with purified bovine 31kD inhibin (10-20µg hormone/1.5-6ml of Dulbecco's Phosphate buffer pH 7.0 (40%) + Montanide 888 : Marcol 52 1:9 (60%)). Following an initial injection and 3-7 boosters the clotting time of the blood collected via the marginal ear vein using the flask suction method (Nerenberg et al, J Imm Meths 24 1978, 19-24) was determined (see Table 2).

TABLE 2

Treatment	Rabbit Number	Clotting time (seconds)*
Immunised	749 & 1989	15 - 60
Controls	1 & 2	120 - 480

*Observations were made by two independent operators on several occasions.

EXAMPLE 5

Tracer Binding Assay

Sheep sera were diluted 1:500 in 0.01M phosphate buffered saline (36g NaCl, 5.52g Na $H_2PO_4 2H_2O$, 0.4g Na Azide in 4 litres of distilled water at pH 7.4, containing 0.5% bovine serum albumin [BSA] referred to as buffer, below) and 100µl of this was added to a plastic tube; 200µl of buffer was also added. To this was added 100µl of approximately

10,000cpm of ^{125}I-31kD purified bovine inhibin. The tubes were incubated overnight at room temperature (14-20°C). A second antibody (anti-sheep IgG raised in donkey) was added, diluted 1:20 with buffer, and added 100µl to each tube then incubated for 30-45 min followed by the addition of 1ml of 6% polyethylene Glycol 6000 (PEG). Tubes were vortexed and centrifuged for 30 min at 2,500rpm. The supernatants were decanted and allowed to drain. Pellets were counted in a multi-channel γ-counter and results expressed as a % of total radioactive counts added (minus NSB) giving % binding of 31kD bovine inhibin to the antibodies in the sera.

EXAMPLE 6

Effects of Recombinant Inhibin α subunit Immunisation on Clotting Time in Adult intact Female Sheep

Nine ewes were immunised with bovine inhibin α subunit fused to part of ß-galactosidase (300µg protein/ml of saline + Montanide 888 : Marcol 52 1:9 (50:50)). Animals were given a primary and booster immunisations (intramuscularly, i. m.) 4 weeks apart and left for several months. Blood (10ml) was collected by vacutainer and the clotting time assessed using a stopwatch. Inhibin antibody titres were determined by tracer binding assay on sera derived from the same sample of blood and animals were placed accordingly into two groups, high (> 1% binding at 1:500 dilution) and low responders (< 1% binding at 1;500 dilution) and compared to the control group.

Immunisation of sheep against inhibin α subunit results in significantly elevated inhibin binding (Figure 6) and faster clotting times (Figure 7). The antibodies are operating as antagonists of inhibin activity and indicate that other chemicals that act as inhibin antagonists and/or other proteins such as activin and TGF-ß with effects known to be opposite to those of inhibin can be administered to achieve the same effect.

INDUSTRIAL APPLICATIONS

The methods of the invention are applicable to the treatment of blood clotting disorders using inhibin or inhibin antagonists or compositions incorporating inhibin or inhibin antagonists.

Delay of clotting or thrombic processes can be achieved by administration of inhibin whereas antagonists to inhibin can be utilised to enhance clotting processes.

These aspects of the invention find utility in the treatment of haemorrhaging and clotting disorders and in cardiovascular therapy in general.

Methods for treating immune dysfunction with inhibin and activin are also described as well as methods for promoting tissue growth using activin.

ACTION OF HORMONES

CLAIMS

1. A method for the treatment of immunosuppressive states or immunodeficiency diseases in a host in need of such treatment, which method comprises administering an effective amount of inhibin or of an inhibin composition to said host.

2. A method for stimulating the immune response of a host in need of such treatment, to an infection or to a tumour, which method comprises administering an immune response stimulating amount of inhibin or of an inhibin composition to said host.

3. A method for the treatment of an autoimmune disease in a host in need of such treatment which method comprises immunising said host with an autoimmune disease treating amount of inhibin, the α subunit of inhibin, or of an inhibin or α subunit composition.

4. A method for suppressing transplantation rejection in a host in need of such treatment which method comprises immunising said host with a transplantation rejection suppressing amount of inhibin, α subunit or of an inhibin or α subunit composition.

5. A method for suppressing transplantation rejection in a host in need of such treatment which method comprises administering a transplantation rejection suppressing amount of an inhibin antagonist or an inhibin antagonist composition to said host.

6. A method for monitoring the immune status of a host in need of such treatment which method comprises measuring the ability of the host's immune system to be stimulated by inhibin, by radioimmunoassay or bioassay.

7. A method for the treatment of an autoimmune disease in a host in need of such treatment which method comprises administering an autoimmune disease treating amount of activin or of an activin composition to said host.

8. A method for suppressing transplantation rejection in a host in need of such treatment, which method comprises administering a transplantation rejection suppressing amount of activin or of an activin composition to said host.

9. A method for the promotion of tissue regeneration in a wound including a surgical lesion, burn, tissue graft or chronic ulcer of a host in need of such treatment, which method comprises administering tissue regeneration promoting amount of activin or of an activin composition to said host.

10. A method for monitoring the immune status of a host in need of such treatment which method comprises measuring the ability of the host's immune system to be depressed by activin by radioimmunoassay or bioassay.

11. A method for monitoring tumour growth in a host in need of such treatment which method comprises measuring the levels of activin in serum or tissue biopsy samples from said host by radioimmunoassay or bioassay.

12. A method for enhancing blood clotting in a host in need of such treatment which method comprises immunsing said host with blood clotting enhancing amount of inhibin or the α subunit of inhibin or of an inhibin composition or of an α subunit composition.

13. A method for enhancing blood clotting in a host in need of such treatment which method comprises administering blood clotting enhancing amount of an inhibin antagonist or an inhibin antagonist composition to said host.

14. A method for inhibiting blood clotting in a host in need of such treatment which method comprises administering a blood clotting inhibiting amount of inhibin or of an inhibin composition, to said host.

15. A method for delaying thrombic processes in a host in need of such treatment, which method comprises, administering a thrombic process delaying amount of inhibin, or of an inhibin composition, to said host.

16. A method for inhibiting excessive tissue proliferation in a host in need of such treatment which method comprises administering an effective amount of an activin antagonist or of an activin antagonist composition to said host.

17. A method of using inhibin in the preparation of a pharmaceutical composition for the delaying of thrombic processes.

18. A method of using an activin antagonist in the preparation of a pharmaceutical composition for the inhibition of excessive tissue proliferation.

19. A method of using inhibin in the preparation of a pharmaceutical composition for the treatment of immunosuppressive states or immunodeficiency diseases.

20. A method of using inhibin in the preparation of a pharmaceutical composition for stimulating the immune response of a host to an infection or a tumour.

21. A method of using inhibin or the α subunit of inhibin in the preparation of an immunogen for protecting a host against an autoimmune disease.

22. A method of using an inhibin antagonist in the preparation of a pharmaceutical composition for protecting a host against an autoimmune disease.

23. A method of using inhibin or the α subunit of inhibin in the preparation of an immunogen for protecting a host against a transplantation rejection response.

24. A method of using an inhibin antagonist in the preparation of a pharmaceutical preparation for protecting a host against a transplantation rejection response.

25. A method of using inhibin in the preparation of a diagnostic agent for monitoring the immune status of a host.

26. A method of using activin for the preparation of a pharmaceutical composition for the treatment of an autoimmune disease.

27. A method of using activin for the preparation of a pharmaceutical composition for suppressing transplant rejection.

28. A method of using activin for the preparation of pharmaceutical composition for promotion of tissue regeneration in a wound, burn, tissue graft or chronic ulcer.

29. A method of using activin for the preparation of a pharmaceutical composition for monitoring the immune status of a host.

30. A method of using activin for the preparation of a pharmaceutical composition for monitoring tumour growth in a host.

31. A method of using inhibin or the α subunit of inhibin for the preparation of an immunogen for enhancing blood clotting.

32. A method of using an inhibin antagonist for the preparation of a pharmaceutical composition for enhancing blood clotting.

33. A method of using inhibin for the preparation of a pharmaceutical composition for inhibiting blood clotting.

34. A method for treatment of an autoimmune disease in a host in need of such treatment which method comprises administering an autoimmune disease treating amount of an inhibin antagonist or of an inhibin antagonist composition to said host.

35. A method for suppressing transplantation rejection in a host in need of such treatment, which method comprises administering, a transplant rejection suppressing amount of inhibin antibodies, α subunit antibodies or of an inhibin or α subunit antibody composition to said host.

36. A method for enhancing blood clotting in a host in need of such treatment which method comprises administering a blood clotting enhancing

amount of inhibin antibodies, α subunit antibodies, an inhibin antibody composition or an α subunit antibody composition to said host.

37. A passive vaccine for treating an autoimmune disease comprising, inhibin antibodies, α subunit antibodies, an inhibin antibody composition or an α subunit antibody composition.

38. A passive vaccine for suppressing transplant rejection, comprising inhibin antibodies, α subunit antibodies, an inhibin antibody composition or an α subunit antibody composition.

39. A passive vaccine for enhancing blood clotting comprising, inhibin antibodies, α subunit antibodies, an inhibin antibody composition or an α subunit antibody composition.

40. A vaccine for treating an autoimmune disease comprising inhibin, the inhibin α subunit, an inhibin composition or an α subunit composition.

41. A vaccine for suppressing transplant rejection comprising inhibin, the inhibin α subunit, an inhibin composition or an α subunit composition.

42. A vaccine for enhancing blood clotting comprising inhibin, the inhibin α subunit, an inhibin composition or an α subunit composition.

43. A method for preventing haemorrhaging in a host in need of such treatment which method comprises administering a haemorrhaging preventing amount of an inhibin antagonist or an inhibin antagonist composition to said host.

44. A method for preventing haemorrhaging in a host in need of such treatment which method comprises immunising said host with a haemorrhaging preventing amount of inhibin, the α subunit of inhibin or an inhibin or α subunit composition.

45. A method of using an inhibin antagonist in the preparation of a pharmaceutical composition for preventing haemorrhaging.

46. A method of using inhibin or the α subunit of inhibin in the preparation of an immunogen for preventing haemorrhaging.

47. A passive vaccine for preventing haemorrhaging comprising inhibin antibodies, α subunit antibodies, an inihibin antibody composition or an α subunit antibody composition.

48. A vaccine for preventing haemorrhaging comprising inhibin, the α subunit of inhibin or an inhibin or α subunit composition.

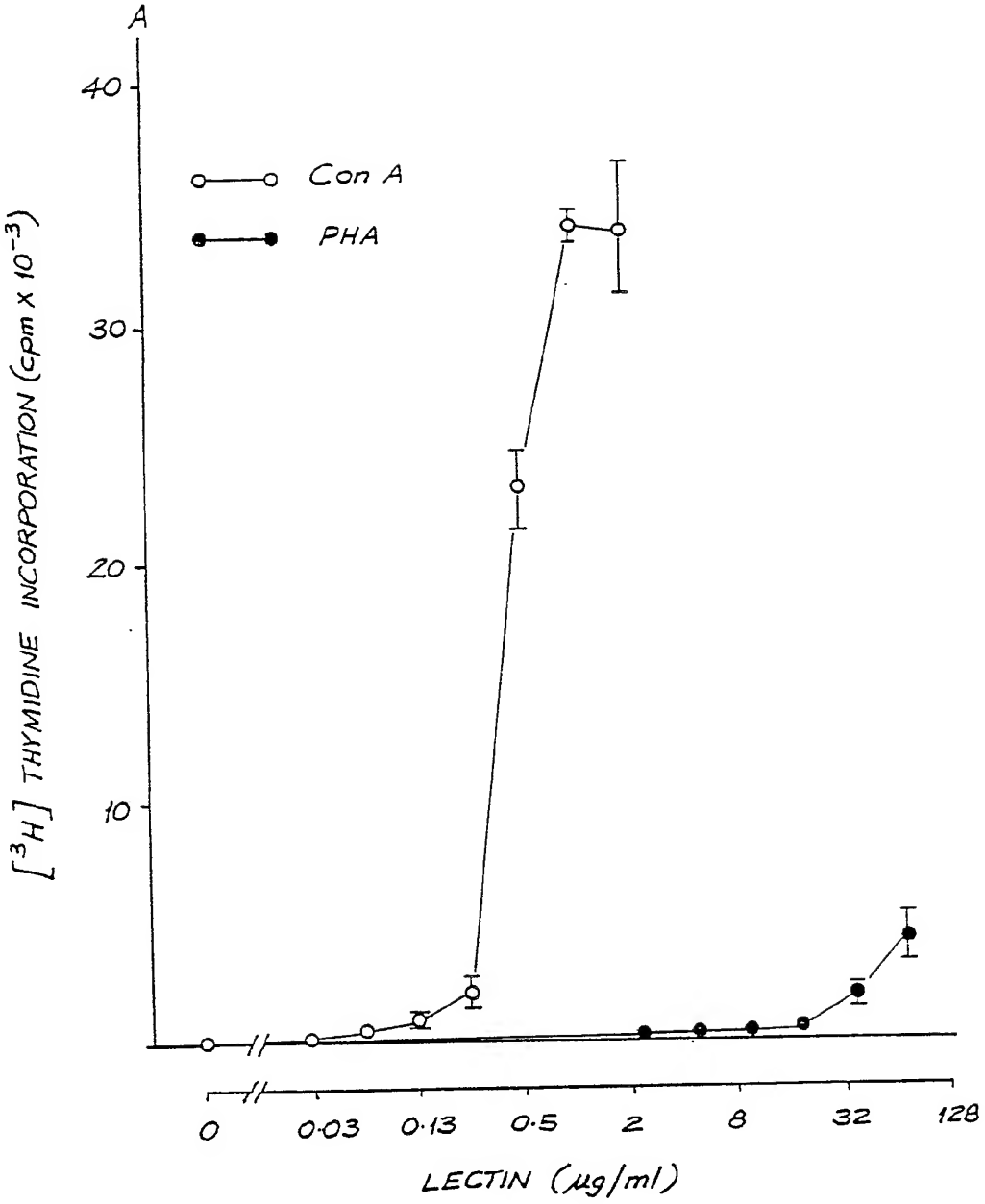
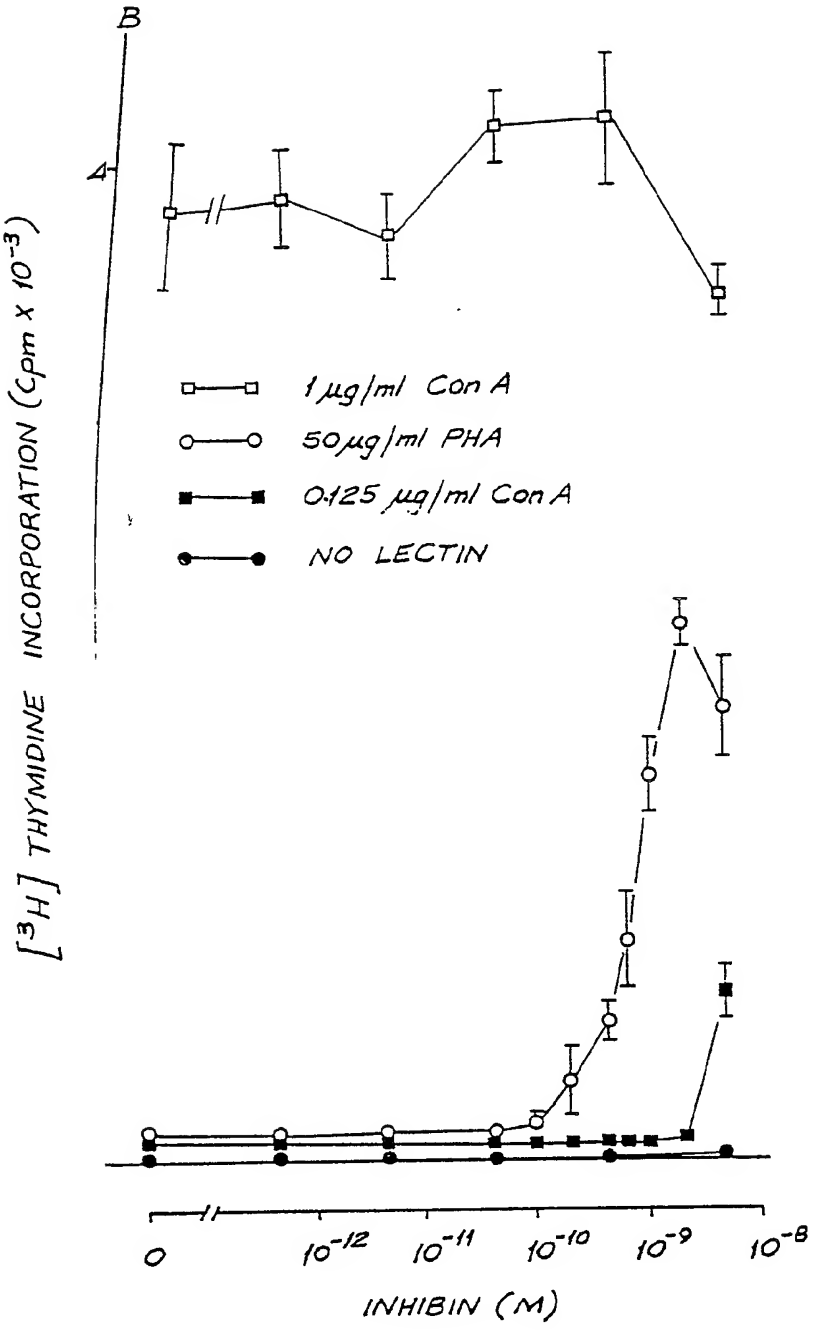

A
40
30
20
10
Con A
PHA
[3H] THYMIDINE INCORPORATION (cpm x 10-3)
0
0·03
0·13
0·5
2
8
32
128
LECTIN (µg/ml)

FIG. 1


B
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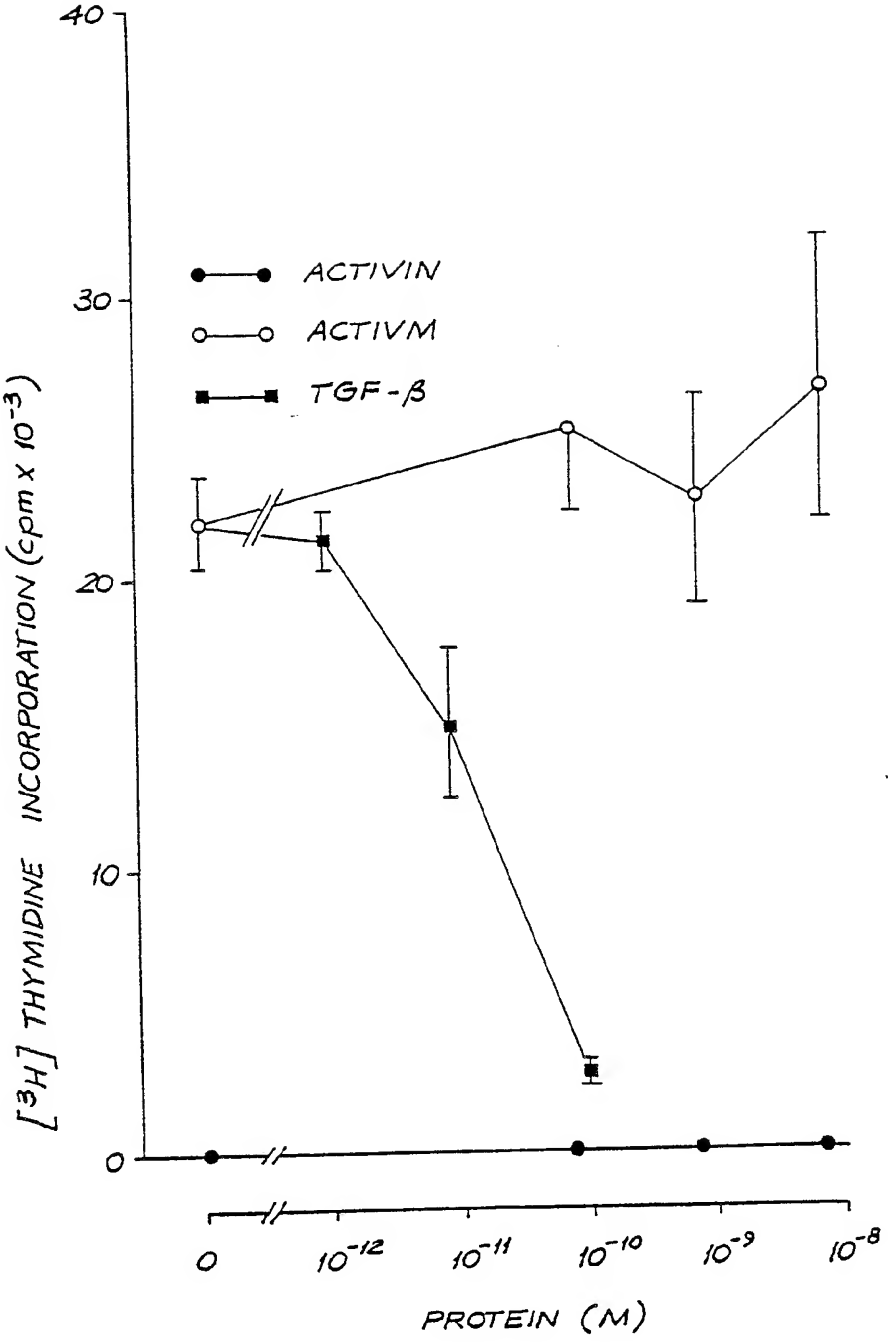
1 µg/ml Con A
50 µg/ml PHA
0.125 µg/ml Con A
NO LECTIN
0
10-12
10-11
10-10
10-9
10-8
INHIBIN (M)

FIG. 1 CONT.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30
20
10
0
ACTIVIN
ACTIVM
TGF-β
[3H] THYMIDINE INCORPORATION (cpm x 10-3)
0
10-12
10-11
10-10
10-9
10-8
PROTEIN (M)

FIG. 2

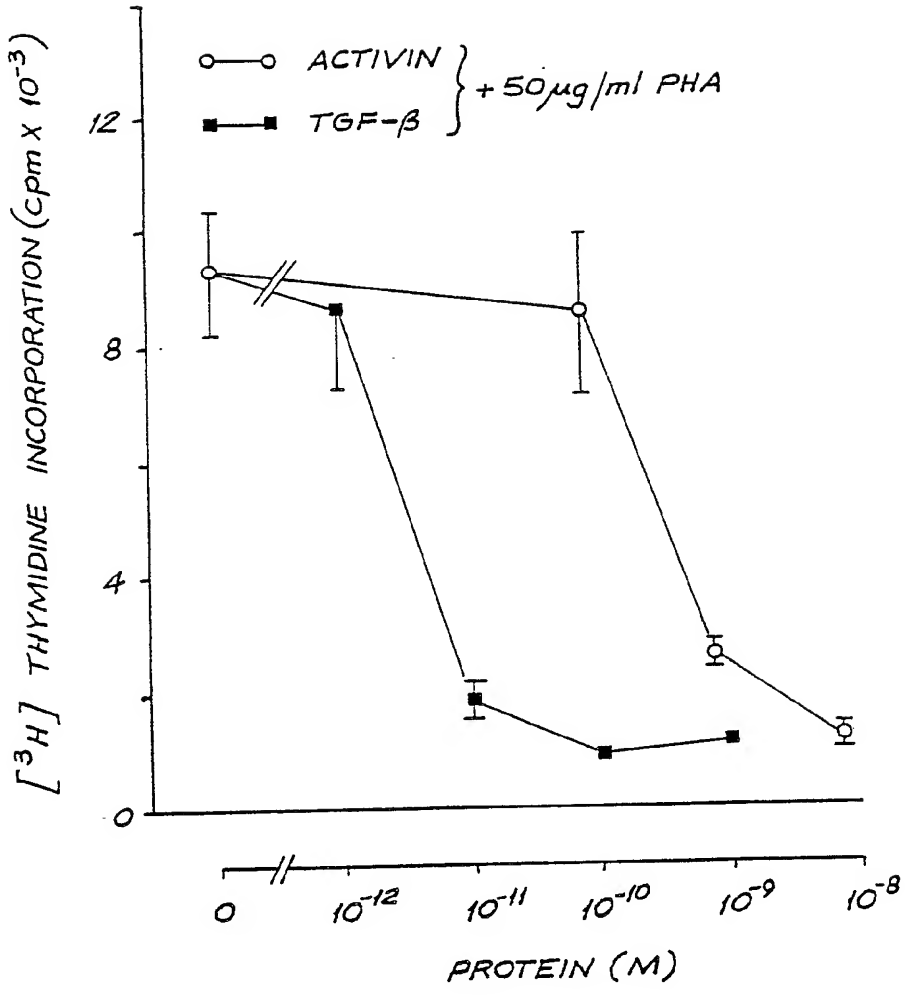
ACTIVIN
TGF-β
+ 50μg/ml PHA
[3H] THYMIDINE INCORPORATION (cpm X 10-3)
12
8
4
0
0
10-12
10-11
10-10
10-9
10-8
PROTEIN (M)

FIG. 3

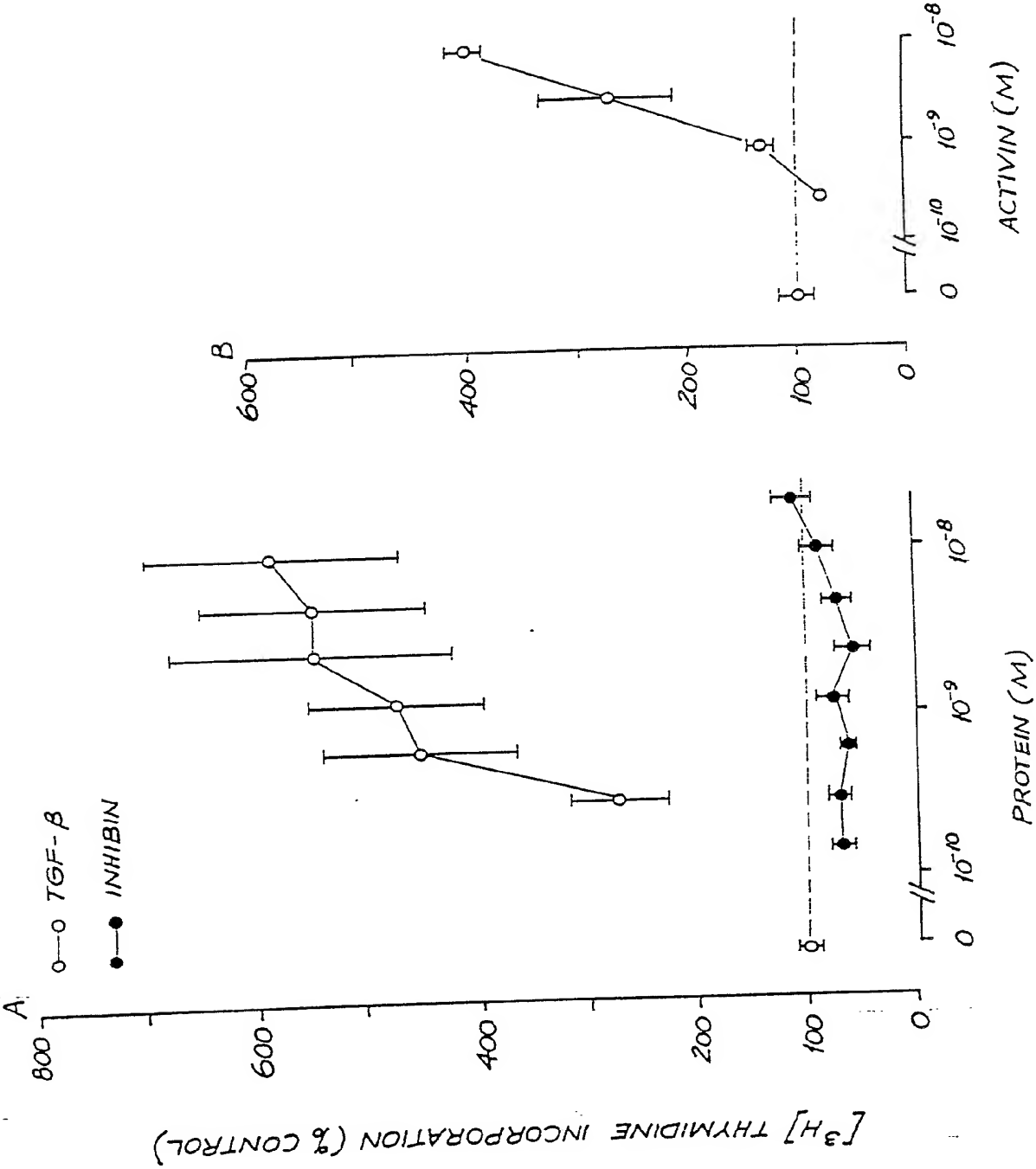
A
B
TGF-β
INHIBIN
[³H] THYMIDINE INCORPORATION (% CONTROL)
800
600
400
200
100
0
PROTEIN (M)
ACTIVIN (M)
10⁻⁸
10⁻⁹
10⁻¹⁰

FIG.

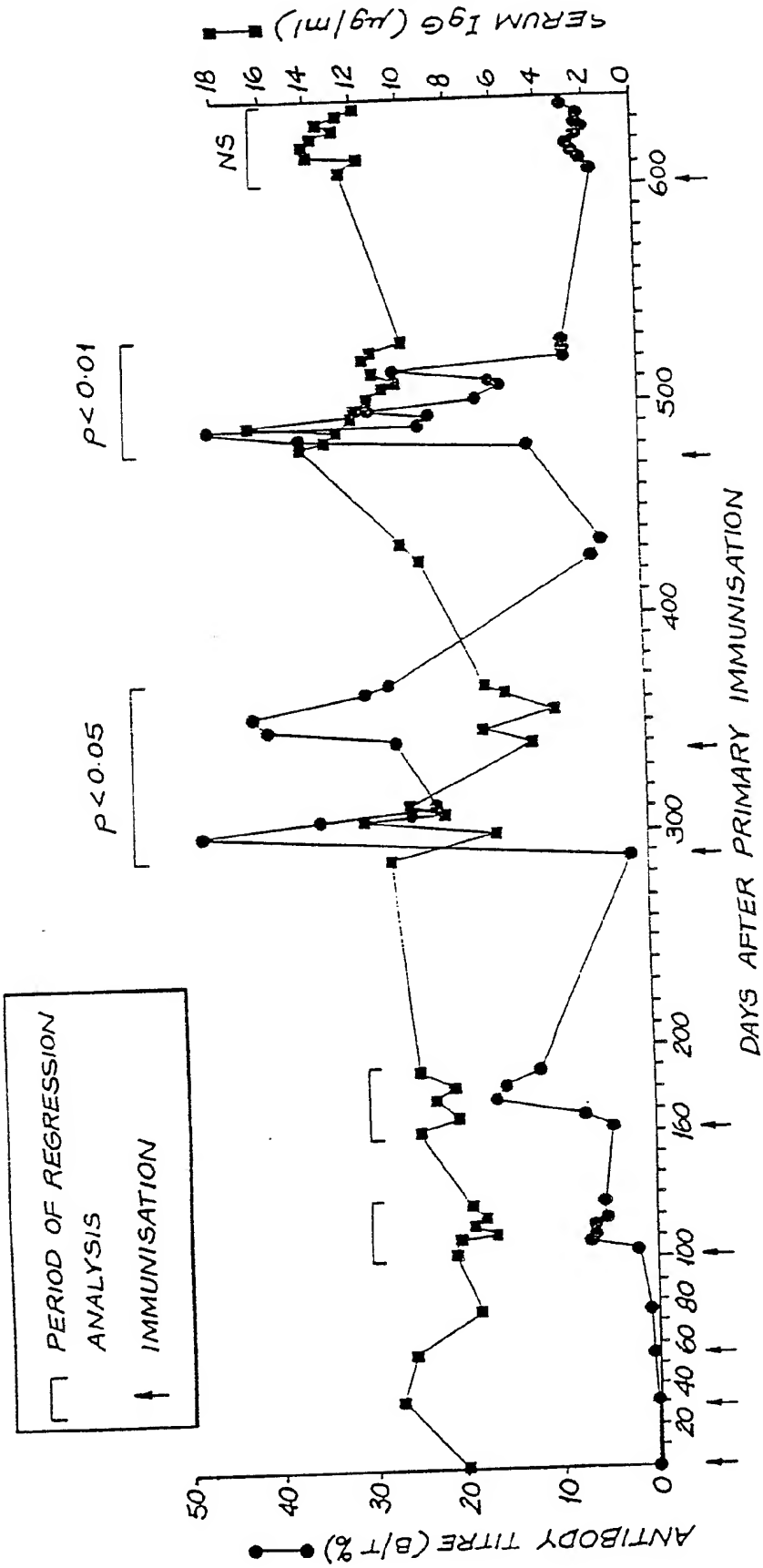
SERUM IgG (μg/ml)
ANTIBODY TITRE (B/T %)
DAYS AFTER PRIMARY IMMUNISATION
PERIOD OF REGRESSION ANALYSIS
IMMUNISATION
NS
P<0·01
P<0·05

FIG. 5A

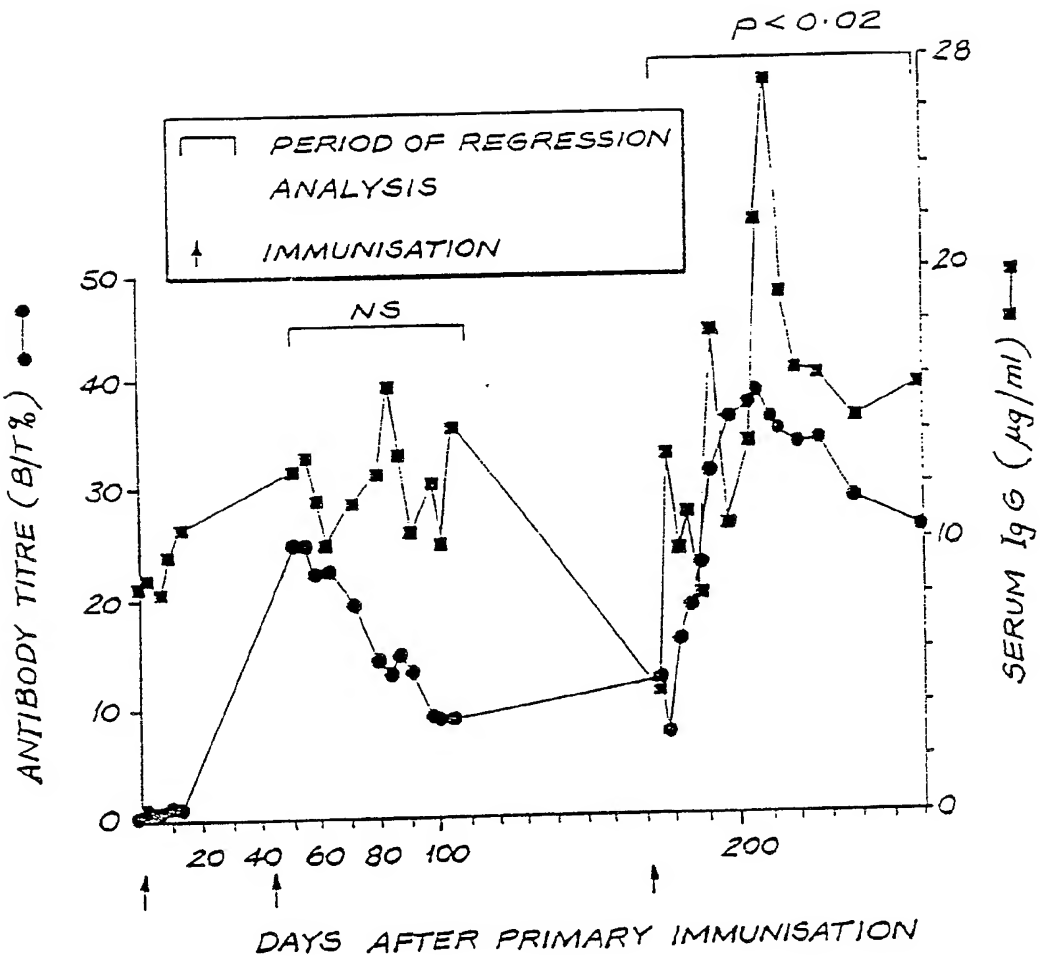
p < 0·02
PERIOD OF REGRESSION ANALYSIS
IMMUNISATION
NS
ANTIBODY TITRE (B/T%)
SERUM IgG (μg/ml)
50
40
30
20
10
0
28
20
10
0
20 40 60 80 100
200
DAYS AFTER PRIMARY IMMUNISATION

FIG. 5B

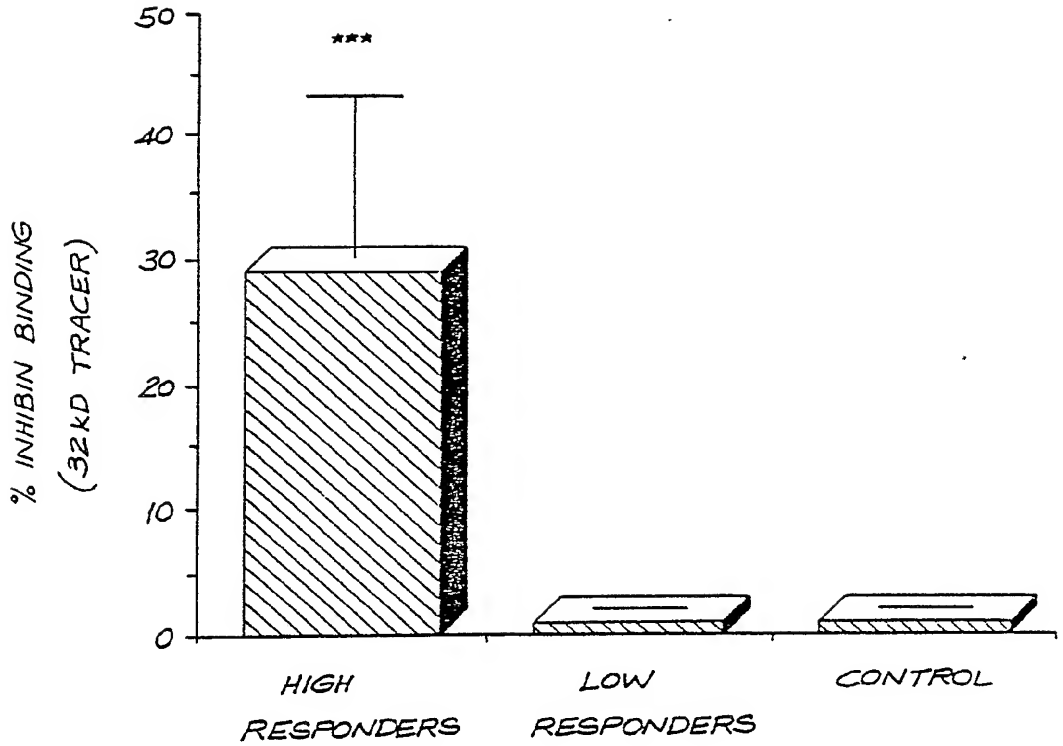
50
40
30
20
10
0
***
% INHIBIN BINDING
(32kD TRACER)
HIGH RESPONDERS
LOW RESPONDERS
CONTROL

FIG. 6

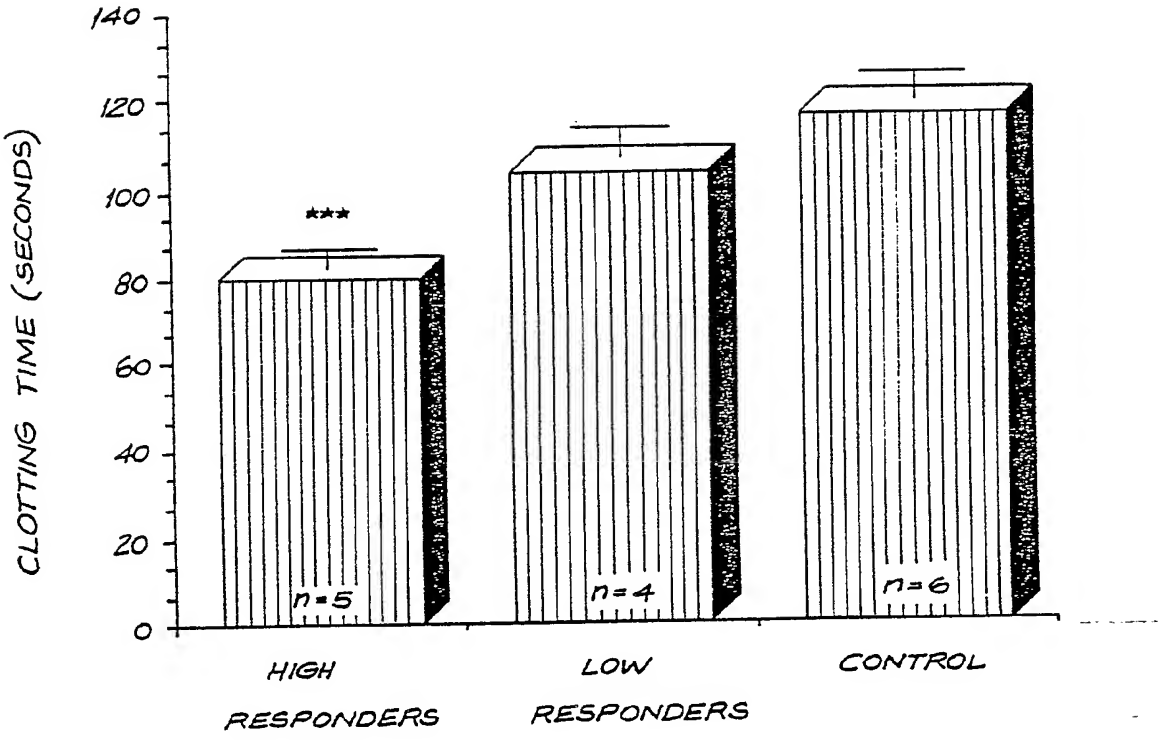
CLOTTING TIME (SECONDS)
140
120
100
80
60
40
20
0
***
n=5
n=4
n=6
HIGH RESPONDERS
LOW RESPONDERS
CONTROL

FIG. 7

INTERNATIONAL SEARCH REPORT

International Application No. PCT/AU 89/00245

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) 6

According to International Patent Classification (IPC) or to both National Classification and IPC

Int. Cl.[4] A61K 37/24, A61K 37/38, C07K 15/06

II. FIELDS SEARCHED

Minimum Documentation Searched 7

Classification System	Classification Symbols
IPC	A61K 37/24, A61K 37/38, C07K 15/06

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched 8

AU: IPC as above

III. DOCUMENTS CONSIDERED TO BE RELEVANT 9

Category*	Citation of Document, 11 with indication, where appropriate, of the relevant passages 12	Relevant to Claim No 13
X	AU,A, 44374/85 (Monash University) 3 January 1986 (03.01.86) See claims 35-40	(37-42, 47-48)
X	AU,A, 59039/86 (Biotechnology Australia Pty Ltd) 23 October 1986 (23.10.86) See claim 54	(37-42, 47-48)
X	AU,A, 71015/87 (Biotechnology Australia Pty Ltd, Monash University, Prince Henry's Hospital, St Vincent's Institute of Medical Research) 25 June 1987 (25.06.87) See claims 55-56	(37-42, 47-48)

* Special categories of cited documents: 10

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
4 September 1989 (04.09.89)	15 September 1989 (15.09.89)
International Searching Authority	Signature of Authorized Officer
Australian Patent Office	JOHN G HANSON

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. [X] OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE 1

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [x] Claim numbers 1-36, 43-46, because they relate to subject matter not required to be searched by this Authority, namely:
These claims are methods for treatment of the human or animal body.

2. [ ] Claim numbers ..., because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claim numbers ..., because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4 (a):

VI. [ ] OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING 2

This International Searching Authority found multiple inventions in this international application as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. [ ] As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

[ ] The additional search fees were accompanied by applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON
INTERNATIONAL APPLICATION NO. PCT/AU 89/00245

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report	Patent Family Members		
AU 71015/87	CN 86103459	DK 6113/86	EP 218717
	IL 78519	JP 62501680	WO 8606076
	ZA 8602848	AU 59039/86	
AU 59039/86	DK 6113/86	JP 62501680	WO 8606076
	AU 71015/87	CN 86103459	EP 218717
	IL 78519	ZA 8602848	
AU 44374/85	JP 61502399	WO 8600078	EP 185034
	IL 75412	NO 860427	ZA 8504346

END OF ANNEX